



Storage Stability of Encapsulated Anthocyanin-Rich Extract from Black Carrot (*Daucus Carota ssp. Sativus*) using different Coating Materials

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Abstract

The enzymatic assisted cum hydraulic pressed extract of black carrot which has high anthocyanin content were encapsulated through spray drier using a mixture of Jack fruit seed starch, Soy protein and NBRE-15 (SET -1) and in the second set of experiment using Jack fruit seed starch, Whey protein and NBRE-15 (SET-2) as coating materials. The quality attributes of the powders which were produced at an optimum mixture of SET -1 and SET-2 were characterized by anthocyanin content, antioxidant capacity and L*, a*, b*, C and H^o value. It was reported that SET-1 was found better-encapsulating material as compared to SET-2. SET-1 had higher retention of anthocyanin content, color, antioxidant activity during storage at 25 °C.



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Soy Protein Isolate (SPI).

Introduction

Anthocyanins are the natural pigment present in many flowers, fruits and vegetables and are chiefly responsible for their red, blue or purple color.¹ Research on the application of anthocyanins, due to their potential health benefits in combating several diseases; have attracted great interest in the functional food, nutraceutical and pharmaceutical industries recently. Antioxidant activity of anthocyanins act as an anticarcinogenic agent and are helpful in treating diseases such as neural and cardiovascular illnesses and diabetes.^{2,3} Most of the industries


are using the synthetic food color have a chemical source that causes adverse health effect. Black carrot anthocyanins can be used as a natural food colorant having antioxidant properties to promote health benefits.⁴ The black carrot was originated from middle Asia and is considered to be the archetype of all modern orange carrots.⁵ Carrot, in terms of their nutritional value among 38 other fruits and vegetables has been ranked tenth.⁶

Five major anthocyanins pigment present in Black carrot are, two nonacylated, cyanidin

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3-xylosylglucosylgalactoside (Cya3XylGlcGal) and cyanidin 3-xylosylgalactoside (Cya3XylGal), and three derivatives of cyanidin acylated with sinapic acid (cyanidin 3-sinapoylxylosylglucosylgalactoside, Cya3SXylGlcGal), ferulic acid (cyanidin 3-feruloylxylosylglucosylgalactoside, Cya3FXylGlcGal) and p-coumaric acid (cyanidin 3-p-coumaroylxylosylglucosylgalactoside, Cya3pCXylGlcGal).⁷ Birks⁸ has been discussed the use of black carrot anthocyanins as the natural colorant in the production of confectionery, jellies, jams and frozen desserts. The factors that affect the color and stability of anthocyanins are structure and concentration, pH, temperature, light, presence of pigments, self-association, metallic ions, enzymes, oxygen, ascorbic acid, sugar and their degradation products, proteins and sulfur dioxide.⁹⁻¹¹

Increasing the demands of nutraceutical food due to increased consumer awareness, researchers are now concentrating to developed food with more functional and nutraceutical properties¹²⁻¹⁹ Among them encapsulation technology is a major area to develop functional ingredients for the fortification in to different food matrices²⁰⁻²⁴ Encapsulation is a technique to prevent the sensitive materials such as anthocyanins from moisture, heat, light or oxidation.^{4,25} It is a packaging technique of materials in the form of micro and nano particles.²⁶ Because of high perishable nature of black carrot, it is difficult to preserve it as a raw product. Spray drying is a new technique to preserve most of the anthocyanins present in the black carrot in combination with carrier agents for a long time. There are several methods for encapsulation of anthocyanins has been described.²⁷⁻³² Spray drying has been used as a simple technique in encapsulating water-soluble essence natural aroma and color.^{4,25,28} Spray dried black carrot anthocyanin have better heat, light and pH stability, therefore, have enhanced shelf life and stability.^{5,6,26,33} The present study was conducted to study the storage stability of spray-dried anthocyanin-rich extract from black carrot encapsulated using.

Materials and Methods

Black carrot samples for anthocyanin extraction were grown in the Division of Vegetable Science, Indian Agriculture Research Institute, New Delhi and transferred to the Division of Food Science and

Post-Harvest Technology, IARI, New Delhi in about 20 kg plastic bags and kept at -20 °C until extraction.

Juice Extraction

Carrots were manually peeled using a stainless-steel knife and crushed coarsely by using a blender (Waring Laboratory Science Torrington, USA). For the inactivation of polyphenol oxidase activity, the crushed mass was heated to 60 °C in a water bath and cooled rapidly to 45 °C with ice water. The pectinase enzyme (EC3.2.1.1 from *Aspergillus niger*, 1U/mg, optimum pH 4.5-5.5) was added to the mass at 0.2 ml/kg mixed thoroughly and incubated at 60 °C for 1 hour. The mass immediately pressed (1800kg/m²) in a hydraulic press (Jonston automation, India). The extracted juice was heated to 90 °C for 1 minute then cool to 5 °C and packed in pre-sterilized amber colored glass bottles.

Carrier Agent for Spray Drying

Jack fruit seed starch (JSS), Whey protein, Soy protein were obtained from the local market of New Delhi and NBRE-15 (type of plant source emulsifier developed in lab, patent filed) was from National Botanical Research Institute, Lucknow.

Preparation of Feed Mixture

A mix of different carrier agents viz., jackfruit seed starch, soy protein and NBRE-15 (SET -1) and jackfruit seed starch, whey protein and NBRE-15 (SET-2) were used to encapsulate the anthocyanin extract. Previously optimized ratio of JSS:SPI: NBRE-15 and JSS:WPI: NBRE-15 were separately used to prepare the anthocyanin feed mixture at the optimized concentration of 27.3 °Brix (SET-1) and 27.8 °Brix (SET-2). Brix was measured after homogenization of the mixture using digital ultraturrax homogenizer (IKT®T25) for the stirring period of 30 minutes.⁴

Spray Drying

The feed mixture was spray dried in a SonoDry 1000 (SONO-TEK, USA) advance laboratory spray drier with complete unit 650mm L×550 mm W×1625 mm H and drying chamber diameter size 230 mm. The operating condition was given in the table -1.

Encapsulated Anthocyanin Powder Storage

Encapsulated black carrot anthocyanins powder were stored in amber colored bottles (ACBs) and in

transparent colored bottles (TCBs) with screw caps and placed at 25 °C to determine the quality attribute of encapsulated anthocyanin powder. Degradation study was followed for 9 weeks of storage and the content was analyzed weekly according.³⁴

Moisture Content of Encapsulated Anthocyanin Powder

The moisture content of the spray dried powder was determined by using a vacuum oven method. 2 g powder samples were measured and kept in the oven at 70 °C and 100 mm Hg vacuum for 7-8 h. After cooling in desiccators, the moisture content in percentage was calculated by using the following equation.

$$\text{Moisture content (\% w.b.)} = \frac{\text{Initial weight (g)} - \text{Final weight (g)}}{\text{Initial weight}} \quad \dots(1)$$

Color Measurement

The color changes in the encapsulated samples were measured with Hunter Lab colorimeter (LabScan® XE Plus 4500 L, USA). The instrument was calibrated with black and white reference tiles taking as a standard. The color value were expressed as L* (lightness/darkness), a* (red/green), b* (yellow/blue), C (Chroma) and H° (hue angle).³⁵

$$C = (\alpha^{*2} + b^{*2})^{1/2} \quad \dots(2)$$

$$H^{\circ} = \tan^{-1} (b^{*}/a^{*}) \quad \dots(3)$$

Total Anthocyanins Content

The total monomeric anthocyanin was determined by pH differential method.³⁶ Two buffer systems were prepared- potassium chloride buffer, pH 1 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M). The pH

was adjusted with HCl. Samples were diluted in pH 1 and pH 5 buffers and the absorbance measurement were made at 510 and 700 nm respectively. Anthocyanins in encapsulated powder expressed as cyanidin-3-glucoside equivalents, as follows:

$$\text{Cyanidin - 3 - glucoside equivalents (mg/L)} = \frac{A \times MW \times DF \times 1000}{(\epsilon \times 1)} \quad \dots(4)$$

Where, A (Absorbance) = pH 1.0 ($A_{510} - A_{700}$) - pH 4.5 ($A_{510} - A_{700}$); MW= Molecular weight (449.2 for cyanidin-3-glucoside); DF= Dilution Factor; ϵ = Molar absorptive (26 900 molar extinction coefficient, in L & mol⁻¹& cm⁻¹ for cyanidin-3-glucoside).

The encapsulation efficiency of the anthocyanin was calculated using previously published method.⁴

Antioxidant Activity

Antioxidant activity in encapsulated black carrot powder was determined by CUPRAC method developed by Apaket *et al.*,³⁷ measures the copper (II) reducing ability of polyphenols, Vitamin C and E. For determination of antioxidant activity 100µl supernatant samples (2-5 g samples were extracted in 10-12 ml 80% ethanol and centrifuge at rpm 10000 for 20 min) were mixed with 1 ml 10⁻² M copper chloride, 1 ml 7.5×10⁻³ M Neocuproine (MW= 208.26 g), 1 ml ammonium acetate buffer (pH 7) and 1 ml distilled water in the test tubes. The final volume reached 4.1 ml. The absorbance of the samples was taken at 450 nm after 30 minutes against a reagent blank in the UV -Vis spectrophotometer (Jasco V-670, Japan). The calculation was done using the following equation:

$$\text{Antioxidant capacity } (\mu \text{ mol Trolox/g}) = \frac{A}{\epsilon \cdot TR} \left(\frac{V_f}{V_s} \right) DF \left(\frac{V}{m} \right) \quad \dots(5)$$

Where, A= Absorbance, ϵ TR= Molar absorptivity of Trolox (1.67×10⁴), V_f = Final volume made (4.1 ml); V_s = Sample volume taken from diluted extract (ml), DF= Dilution factor, m= weight of the sample (g)

Result and Discussion

The initial moisture content of spray dried black carrot powder was 4.36 % and 5.02 % for the SET-1 and the SET-2 respectively. It was found that increase in feed flow rate from 2 to 2.5 ml/ min, the moisture content in the encapsulated powder were also

Table 1: Operating conditions of Spray drier

Parameters	Set Conditions
Inlet temperature	150 °C±2 (Minimum), 180±2 (Maximum)
Outlet temperature	50 °C±2 (Min), 80°C±2
Sample temperature	30 °C±2
Aspirator air flow rate	50 m ³ /h
Feed flow rate	2-2.5 ml/min
Compressor pressure	115 psi

increased and reached 4.85 % for SET-1 and 5.42 % for the SET-2. Ersus and Yurdagel³⁸ were also reported that increasing in feed flow rate, increase the moisture content.

During the storage period the L* values increase significantly for SET-1 and for SET-2, however, values of a* and b* declined with respect to days interval. The same results were reported by Kamiloglu *et al.*³⁵ Fresh encapsulated black carrot showed L*, a* and

b* values of 32.20, 28.21 and -1.60 for the SET-1. However, for the SET-2 these values were reported to be 34.32, 24.21 and -1.66 respectively. On the other hand, the C values were 30.77 for SET-1 and 29.96 for SET-2 respectively. Our samples had a negative H° value which ranged -17.61 (for SET-1) to -14.56 (for SET-2) corresponding to a bluish hue for the fresh encapsulants. It was observed that L* values increased as the a* and b* values declined every week during storage. The decline in a* values was

Table 2: Changes in the color of SET-1 during storage

Days	SET-1 (ACBs)					SET-1 (TCBs)				
	L*	a*	b*	C	H°	L*	a*	b*	C	H°
0	32.20	28.21	-1.60	30.77	-17.61	32.20	28.21	-1.60	30.77	-17.61
7	32.25	28.11	-1.80	31.32	-15.59	34.60	27.02	-1.82	30.33	-14.82
14	32.33	28.01	-2.07	32.29	-13.50	34.64	26.65	-2.09	31.01	-12.72
21	32.41	27.85	-2.26	32.95	-12.29	34.66	26.52	-2.31	31.85	-11.45
28	32.60	27.61	-2.38	33.27	-11.57	34.72	26.31	-2.66	33.38	-9.85
35	32.71	27.40	-2.60	34.16	-10.50	34.78	25.73	-2.81	33.62	-9.12
42	32.80	27.33	-2.78	35.05	-9.79	34.83	25.68	-2.96	34.44	-8.63
49	33.02	27.30	-2.94	35.94	-9.24	35.02	25.88	-3.09	35.42	-8.33
56	33.12	27.12	-3.08	36.60	-8.76	35.38	26.04	-3.18	36.15	-8.14
63	33.23	27.02	-3.28	37.77	-8.19	35.51	26.19	-3.30	37.08	-7.89

Note ACBs (Amber colored bottles), TCBs (Transparent colored bottles).

Table 3: Changes in the color of SET-2 during storage

Days	SET-2 (ACBs)					SET-2 (TCBs)				
	L*	a*	b*	C	H°	L*	a*	b*	C	H°
0	34.32	24.21	-1.66	26.96	-14.56	34.32	24.21	-1.66	29.95	-14.56
7	34.55	24.55	-1.91	28.19	-12.82	34.66	24.74	-1.98	28.66	-12.46
14	34.84	24.67	-2.11	29.12	-11.66	34.95	25.02	-2.18	29.77	-11.44
21	34.98	24.79	-2.28	29.98	-10.84	35.11	25.28	-2.36	30.84	-10.68
28	35.31	24.98	-2.45	30.98	-10.16	35.45	25.55	-2.51	31.85	-10.14
35	35.42	25.26	-2.67	32.38	-9.42	35.8	25.79	-2.75	33.35	-9.34
42	35.87	25.41	-2.88	33.70	-8.78	35.97	25.94	-2.89	34.29	-8.93
49	35.37	25.64	-3.02	34.76	-8.45	36.12	26.10	-3.02	35.22	-8.60
56	33.12	27.12	-3.08	36.60	-8.76	35.38	26.04	-3.18	36.15	-8.14
63	33.23	27.02	-3.28	37.77	-8.19	35.51	26.19	-3.30	37.08	-7.89

Note ACBs (Amber colored bottles), TCBs (Transparent colored bottles).

an indication of the reduction of anthocyanin content and shifted towards bluish hue with a negative H° value for both the sets. SET-1 had higher retention of a^* values as compared to SET-2, showing it had a higher capacity to prevent anthocyanin reduction during storage (table 1 and 2). Amber colored bottles (ACBs) were found to be more effective to prevent color degradation over transparent colored bottles (TCBs). The samples had to fall b^* value and attained a higher negative value during week intervals, indicated increased in blueness. Skerede³⁹ reported a decrease in the b^* factor of black carrot syrups during storage.

The anthocyanin content was highly influenced by different coating agents. In case of SET-1 anthocyanin

content was found to be maximum 2969.66 mg/100 g of dry matter with encapsulation efficiency 87.72%. On the other hand, SET-2 had low encapsulation efficiency i.e. 75.36% with anthocyanin content 2752.91 mg/100 g of dry matter (Fig. 1). Ersus and Yurdagel³⁸ were reported anthocyanin content in black carrot 2721.61 ± 5.92 mg/100 g of dry matter.

ACBs were reported to be more effective in retaining anthocyanin content over TCBs during storage (Fig. 2). At the end of the storage period, anthocyanin retention in SET-1 and SET-2 stored in ACBs were found 49.98 % and 38.04 %. However, those stored in TCBs had retention of 35.15 % for SET-1 and 34.56 % for SET-2. Fig. 1 and Fig. 2 were showing the reduction pattern in anthocyanin content during storage in TCBs and ACBs.

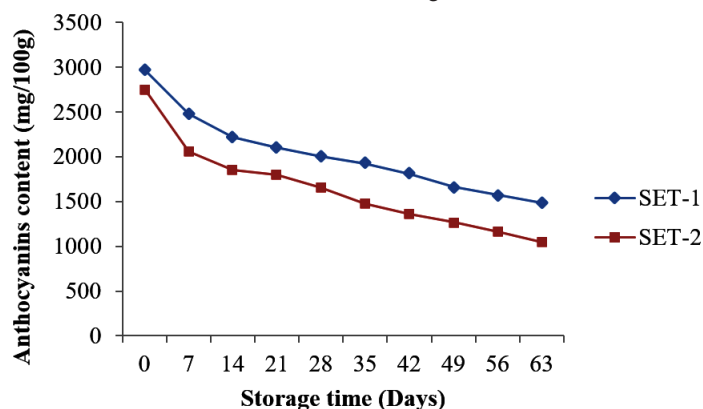


Fig. 1: Anthocyanin content of SET-1 and SET-2 stored in amber colored bottles

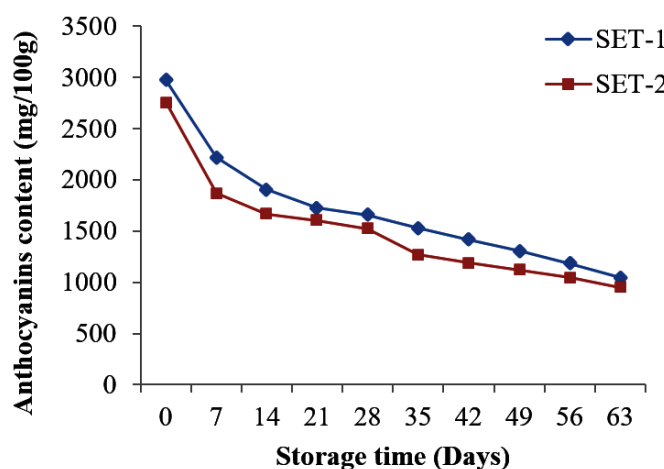


Fig. 2: Anthocyanin content of SET-1 and SET-2 stored in transparent bottles

The reduction in the anthocyanin content may be due to the presence of heat stable form of polyphenol oxidase or peroxidase.^{4,33} It may also a result of the conversion of anthocyanin glycosides to chalcones, resulting in phenolic acids and aldehydes due to hydrolytic reactions.³⁵ Black carrots contain cyanidine based anthocyanin and have vicinal hydroxyl groups. Anthocyanin of this group is expected to more prone to degradation.³³ During storage at 25 °C, the soy protein (SET-1) was reported to be better encapsulating agent than whey (SET-2) containing the encapsulating agent.

Structure plays a key role in the determination of the antioxidant activity of anthocyanins and highly correlated with anthocyanin content and total phenolic compound of food products.^{4,16} The antioxidant activity of encapsulated black carrot was reported to be 344.3 μmol trolox/100g dry matter (for SET-1) and 282.3 μmol trolox/100g dry matter (for SET-2) by using the CUPRAC method. Algarra⁴⁰ were reported antioxidant activity in black carrot by using DPPH and FRAP methods in their study, were low (17.6–240 and 86.4–182 μMTE /100 g fw for DPPH and FRAP, respectively) as compared to

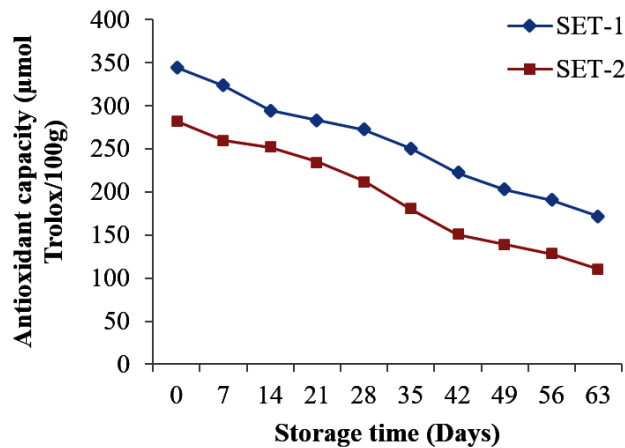


Fig. 3: Antioxidant activity of encapsulated Black carrot (SET-1 & SET-2) stored in amber colored bottles

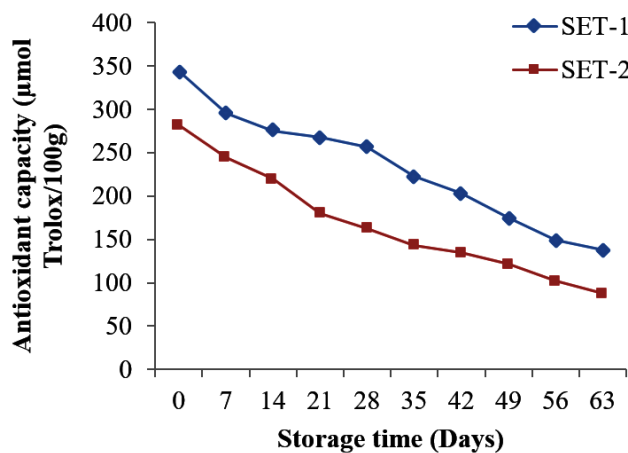


Fig. 4: Antioxidant activity of encapsulated Black carrot (SET-1 & SET-2) stored in transparent colored bottles

our samples. Kamiloglu³⁵ reported antioxidant activity (1720±149 and 5617±357 mg TE/100 g DW for DPPH and FRAP, respectively). The differences in the antioxidant activity depend on various factors like climatic conditions, cultivar, growth conditions and stage of harvesting. The storage temperature plays an important role in determining the stability of the antioxidant. The encapsulated black carrots powders were stored at 25 °C, thus a rapid reduction in the antioxidant activity during storage (Fig. 3 & Fig. 4).

Kamiloglu *et al.*³⁵ were also in the agreement that the more loss in antioxidant activity occurred in black carrot jam and marmalade stored at 25 °C as compared to those stored at 4 °C. The reduction in the antioxidant activity could be due to the presence of oxygen in the headspace of the bottles. At the end of storage, SET-1 showed higher retention i.e. 50% in the antioxidant activity as compared to SET-2 with maximum retention 38.04 stored in ACBs. On the other hand, samples stored in TCBs had retention of 40.14% and 30.93% for SET-1 and SET-2 respectively. The reduction in the antioxidant could also be due to exposure of the samples in the light (for ACBs).

Conclusion

For spray drying black carrot anthocyanin, SET-1 containing soy protein with jack fruit seed starch and NBRE-15 gave highest anthocyanin content and antioxidant capacity powder after drying. In

order to minimize the color loss, anthocyanin content and antioxidant activity these products should be stored in amber colored bottles with minimum head space. SET-1 had higher efficiency to prevent the loss of color, anthocyanin and antioxidant activity during storage.

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Ethical Approval

This article does not contain any studies with either animals or human participants performed by any of the authors.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors contributions

Mr. Brij Bhushan Mishra conducted the analysis of collected samples. Mr. Avinash Singh Patel conducted the design of the experiments and supported Mr. Mishra during analysis. Mr. Mishra has written the manuscript. Dr. Abhijit Kar interpreted the experimental data and reviewed and revised the manuscript.

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